Retention of Aliphatic Alcohols by Anhydrous Lactose

Frederick W. Parrish,* Philip E. Pfeffer, Kenneth D. Ross, Daniel P. Schwartz, and Kathleen M. Valentine

The retention of aliphatic alcohols by anhydrous α-lactose, prepared from α-lactose monohydrate by treating with the appropriate alcohol or by heating α-lactose monohydrate, has been measured by gas chromatographic and by proton magnetic resonance methods. The results are compared with those reported for retention of alcohols by adsorption from the vapor phase or by freeze-drying of aqueous alcoholic solutions. The retention decreases in the order methanol > ethanol > 1-propanol = 1-butanol. β-Lactose prepared by crystallization from aqueous solution does not take up methanol, whereas β-lactose prepared by anomerization of α-lactose in methanol or ethanol retains these alcohols at levels comparable to those found for α-lactose.

Ross (1978) reported that methanol treatment altered several physical properties of lactose, including melting point, heat of fusion, heat capacity, and density. Furthermore, the anhydrous form of α-lactose, α_M, prepared by refluxing α-lactose monohydrate in absolute methanol (Lim and Nickerson, 1973), was shown to differ from α-lactose monohydrate and from the stable, anhydrous species, α_S, produced by heating in air (Sharp, 1943). Two facts argued against the presence of adsorbed methanol in α_M: the samples had been dried to constant weight at 60 °C under vacuum, and no desorption peak was detected in the differential scanning calorimetry (DSC) thermograms, whereas even the tightly held water of crystallization desorbs from α-lactose monohydrate with a characteristic endothermic peak (Berlin et al., 1971). Nevertheless, we thought that the presence or absence of methanol in α_M should be confirmed directly. Other alcohols which are known (Nickerson and Lim, 1974) to remove the water of hydration from α-lactose monohydrate were also included in the study. We have designated the products of treating α-lactose monohydrate with ethanol, 1-propanol, and 1-butanol as α_E, α_P, and α_B, respectively.

MATERIALS AND METHODS

Chemicals. The lactose samples used in this study were prepared by treating α-lactose monohydrate (Sigma Chemical Company, St. Louis, Mo.) with various alcohols at reflux temperature for 2 h or at 27 °C for 16 h. After being cooled to room temperature, the lactose was removed by filtration, washed with the appropriate alcohol, and then dried in a vacuum oven (9 mmHg) at 60 °C until the change in weight of a 15-g sample was less than 1 mg. Constant weight was attained in 24 to 48 h provided the sample thickness did not exceed 1 cm. Lactose samples prepared from the different alcohols were dried separately in order to prevent contamination by another alcohol.

Anhydrous α-lactose, α_S, prepared from α-lactose monohydrate by heating in air in 130 °C for 3 h (Sharp, 1943), was treated similarly with the various alcohols.

Hygroscopic α-lactose, α_H, prepared from α-lactose monohydrate by heating in vacuum at 130 °C for 16 h (Herrington, 1948), and β-lactose, prepared by crystallization from boiling, aqueous solution (Buma and van der Veen, 1974), were treated with methanol. β-Lactose was also prepared by anomerization of α-lactose monohydrate with potassium methoxide in methanol or ethanol (Parrish et al., 1978).

Portions of the alcohol-treated lactose samples were heated in air at 130 °C for 16 h.

Analytical Procedures. Purity of lactose samples was determined by measuring the optical rotation of replicate solutions in water (2–5%) with a Perkin-Elmer Model 141 automatic polarimeter. Calculations were based on the latest available specific optical rotations at 589 and 546 nm (Buma and van der Veen, 1974).

Purity was also determined from the shape of the DSC fusion endotherms at 1 °C/min programming rate (Sondack, 1972). A DuPont Model 990 Thermal Analyzer was used for these determinations, as well as for measurements of melting points. Procedures for instrument

Eastern Regional Research Center, Federal Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

Table I. Melting Points and Purity of Lactose Samples Prepared by Heating α-Lactose Monohydrate

heated in	dried at 60 °C/vacuum			dried at 130 °C/air		
	mp	purity,[a] %	anomeric purity,[b] %	mp	purity,[a] %	anomeric purity,[b] %
unheated control	216	99.9	97.7			
air at 130 °C				216	99.9	91.5
vacuum at 130 °C	216	99.9	91.4			
methanol	210	98.6	97.6	213	99.0	97.8
ethanol	214	99.5	97.7	214	99.6	97.6
propanol	216	99.7	97.7	216	99.7	97.8
butanol	215	99.6	97.7	216	99.7	97.7

[a] As total anhydrous lactose, by polarimetry. [b] As α-lactose, by gas–liquid chromatography.

calibration and specification of melting point have been described previously (Ross, 1978). In addition, large samples (20 mg) of treated lactose were run at high sensitivity in order to detect alcohol desorption peaks and mass loss before melting. These samples were weighed initially and after heating to temperatures just below and just above the onset of fusion.

Pyruvic acid chloride 2,6-dinitrophenylhydrazone was used to form ester derivatives of the alcohols associated with the lactose samples for the purpose of qualitative and quantitative analyses (Schwartz, 1970).

Alcohols in lactose samples were determined by gas–liquid chromatography with a Hewlett-Packard Model 5750A gas chromatograph with a flame ionization detector. Lactose (with associated alcohol) solutions were made by heating, with stirring, an accurately weighed sample (1.5–3.0 g) of lactose with distilled water (7.5 mL) in a sealed 50-mL ampule. When the lactose (with associated alcohol) had dissolved, the solution was cooled to room temperature, transferred to a 10-mL volumetric flask, and made to 10 mL. Injections over the range of 3–11 μL were made onto a silanized stainless steel column (6 ft × 0.25 in.) packed with uncoated Chromosorb 101 (60–80 mesh) previously conditioned 16 h at 222 °C. Helium (30 mL/min) was used as carrier gas. Other conditions were: injection port temperature 120–145 °C; detector, 240 °C; and column temperatures 115 °C for methanol and ethanol, 140 °C for 1-propanol, and 150 °C for 1-butanol. The amount of alcohol in a lactose sample was determined from a standard curve established for solutions of known concentration of pure alcohols in water. Peak sizes were measured on a Digital Readout System Model CRS 11-HSB (Infotronics, Houston, Tex.).

Proton magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL FX-60 Q pulsed Fourier transform spectrometer. Solutions of 0.5 M lactose (with associated alcohol) in deuterium oxide at mutarotational equilibrium were examined with twenty-five 90° pulses with a repetition time of 30 s to ensure complete nuclear relaxation. A sweep width of 1200 Hz and 8K data points were used in all experiments. Shifts were measured relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. The areas of the methyl resonances for both methanol and ethanol at δ 3.60 and 1.83, respectively, were measured by the computer relative to the area of the α-anomeric proton resonance of lactose (relative area of 0.38 proton) at δ 5.21; in the case of methanol-treated samples, a minor contribution to the methyl resonance from lactose was subtracted by use of the computer-stored lactose spectrum (Figure 1). The relative weight percent of alcohol to lactose was calculated from the above peak areas. Measurements were made in the same manner for standard solutions of lactose containing additions of 0–1 wt % of methanol or ethanol to assess the accuracy of the procedure.

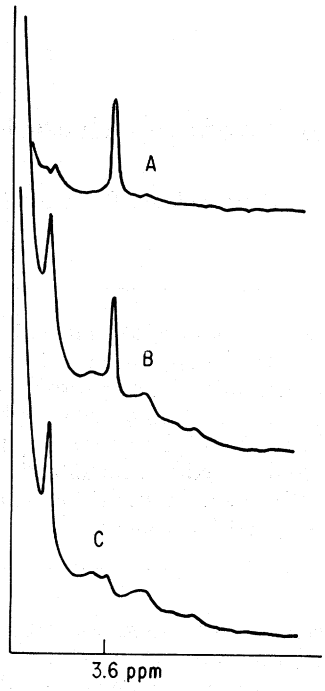

Figure 1. Upfield portion of 60-MHz ^{1}H NMR spectra of (A) computer subtraction of α-lactose monohydrate from α_M, (B) α_M, and (C) α-lactose monohydrate.

Measurement of amounts of alcohols below 0.1 wt % was performed by the inversion recovery (180°–τ–90°) technique with τ = 0.75 s and repetition times of 30 s whereby the areas of the inverted methanol (CH_3) or glycerol (CH_2OH) resonances could be readily evaluated; in the normal spectra, amounts of alcohols below 0.1 wt % were determined with difficulty.

Moisture was determined by the Karl Fischer titration procedure employed for liquid molasses (AOAC, 1975).

The relative amounts of α- and β-lactose were determined by gas chromatography of trimethylsilyl ether derivatives (Buma and van der Veen, 1974).

RESULTS AND DISCUSSION

Polarimetric Analysis. The equilibrium optical rotation at 589 nm and at 546 nm showed that α_M contained 98.6 ± 0.3% total anhydrous lactose, based on accurate values reported recently (Buma and van der Veen, 1974). This purity value is considerably lower than that obtained for the monohydrate used as the starting material and for the other species of lactose reported previously (Ross, 1978). The monohydrate and the other forms of lactose were found to be >99% pure (as total anhydrous lactose) by polarimetric analysis (Table I). No additional formation of β-lactose resulted from alcohol treatment as judged by gas chromatographic analysis (Buma and van der Veen, 1974).

Calorimetry. The level of β-lactose impurity in α_M was found by mathematical analysis of the differential scanning calorimetry (DSC) curve (Sondack, 1972) to be 2.40 wt %, compared with 2.25 wt % obtained for α-lactose monohydrate and α_S. The impurity levels in α-lactose monohydrate and α_S reflect the presence of β-lactose trapped within the crystal structure and not removable by standard purification techniques (Buma and van der Veen, 1974). Since there is no increase in the level of entrapped β-lactose after methanol treatment, the increased impurity content could be the result of retention of methanol. In support of this interpretation is the observation that the first deviation from the baseline in fusion endotherms of α_M is at a much lower temperature than that seen for other species of lactose. That is, there is a broad initial phase of the fusion process, during which a relatively small amount of energy is absorbed (Ross, 1978).

In order to determine if some mass was lost during the initial phase of the fusion endotherm, 20-mg samples of α_M were heated at 10 °C/min from room temperature to the onset of fusion and then slightly above the onset temperature. In no instance was there any indication of a desorption endotherm, or a mass loss of more than 0.01 mg up to the onset of fusion. Above the onset temperature, but below the temperature range of rapid fusion, the mass loss was approximately 1–2% of the initial sample mass.

Calorimetric experiments were performed on 20-mg samples of freshly prepared α_M, α_E, α_P, and α_B before the customary drying step to reach constant weight. Each of these samples showed small endotherms in the range of 25–60 °C, which we attribute to the desorption of excess, surface-bound alcohol.

The results of the above calorimetric experiments suggested that any alcohol incorporated into the species α_M, α_E, α_P, and α_B must be so tightly bound to the lactose that no desorption takes place until the material begins to melt. The effect of the various alcohols on the apparent melting point of α-lactose is presented in Table I. The greatest melting point depression is obtained with α_M, whereas α_P and α_B show little, if any, change from α_S; the value for α_E falls between those of α_M and α_S. Those samples prepared with α_S as starting material and the appropriate alcohol in every case had fusion endotherms indistinguishable from α_S.

Assuming that melting depression is indicative of the level of alcohol incorporation into the lactose structure, then the data suggest that extended exposure to temperatures of at least 130 °C is sufficient to remove some of the incorporated alcohol. The rate of loss of alcohol may be so slow that the DSC thermogram does not detect any desorption until the temperature program has already reached the melting point.

Phenylhydrazones. Qualitative indication of retention of alcohols by the resulting anhydrous α-lactose was obtained by formation of the pyruvate 2,6-dinitrophenylhydrazone esters of the alcohol, and the alcohol was identified by thin-layer chromatographic comparison with authentic derivatives (Schwartz, 1970). Attempts to use this reaction for quantitative analyses of the alcohol contents of the lactose samples proved unsatisfactory, the measured percentage of alcohol decreasing with increasing sample size. No solvent for lactose, e.g., *N*,*N*-dimethylformamide or methyl sulfoxide, which would enable ester formation to be performed in homogeneous solution, was found suitable because of extremely high blank values.

Alcohol Determination by Gas Chromatography or Proton Magnetic Resonance (^{1}H NMR). Gas chromatographic data for the retention of the various alcohols by α-lactose monohydrate and by α_S following heating with these alcohols and after drying at 60 °C in vacuo or at 130 °C in air are shown in Table II. The order of decreasing retention of alcohol by the products derived from α-lactose monohydrate is MeOH > EtOH > PrOH = BuOH. Removal of the retained alcohol by heating in air at 130 °C for 16 h, conditions which readily remove water of crystallization from α-lactose monohydrate, was incomplete. We therefore do not consider that the alcohols are bound in the lactose crystal in the same manner as is water of crystallization. This conclusion is also supported by the shape of the DSC thermograms. Tenacious retention of ethyl alcohol by maltodextrin during drying of aqueous solutions (Menting and Hoogstad, 1967) and of various alcohols by lactose and other carbohydrates during freeze-drying (Flink and Karel, 1970) has been reported, and it was shown that retention was not due to adsorption. An indication that retention in our system was not due to adsorption is that methanol retention in different mesh sizes of α-lactose monohydrate (over 20 mesh, 20–40 mesh, ..., through 100 mesh) showed no significant differences. Furthermore, treatment of α-lactose monohydrate with methanol containing 0, 1, 3, and 5% water gave methanol retentions of 0.86, 0.27, 0.11, and 0.00 wt %, respectively; the extent of retention of methanol correlated with the conversion of α-lactose monohydrate to anhydrous α_M, none of the latter being formed in 95% aqueous methanol (Lim and Nickerson, 1973). No differences in alcohol retention were found when samples were prepared at reflux or at 27 °C. Similar results for retention of methanol and ethanol were obtained by ^{1}H NMR analysis.

When mixtures of methanol and ethanol, or of methanol and ethyl acetate, were used, retention of methanol and

Table II. Gas Chromatographic Analysis of Retention of Alcohols from Treatment of α-Lactose Monohydrate or Anhydrous α-Lactose

	alcohol content in wt %			
	α-lactose monohydrate		anhydrous α-lactose (α_S)	
alcohol	dried at 60 °C	dried at 130 °C	dried at 60 °C	dried at 130 °C
MeOH	0.77 (±0.05)	0.28 (±0.05)	0.14 (±0.02)	0.08 (±0.01)
EtOH	0.42 (±0.01)		0.04 (±0.01)	
PrOH	0.13 (±0.01)	0.10 (±0.01)	0.05 (±0.00)	
BuOH	0.17 (±0.03)	0.10 (±0.00)	0.08 (±0.01)	0.06 (±0.00)

Table III. Proton Magnetic Resonance Analysis of Retention of Alcohols after Treatment of α-Lactose Monohydrate

	alcohol content in wt %	
solvent	MeOH	EtOH
MeOH	0.79 (±0.03)	
EtOH		0.43 (±0.03)
MeOH/EtOH (1.45:1)[a]	0.35 (±0.00)	0.11 (±0.01)
MeOH/EtOH (1:3)[a]	0.11 (±0.01)	0.20 (±0.02)
MeOH/EtOAc (1.61:1)[a]	0.22 (±0.01)	
MeOH/EtOAc (9.66:1)[a]	0.40 (±0.01)	
MeOH/KOMe	0.79 (±0.03)	
EtOH/KOMe		0.44 (±0.02)

[a] Mole ratio.

ethanol, but not of ethyl acetate, was found (Table III). The data show preferential retention of methanol over ethanol; however, treatment of α_M or α_E with ethanol, methanol, or 90% alcohol did not cause displacement or result in incorporation of either alcohol. Removal of the water of crystallization from α-lactose monohydrate occurred with ethyl acetate or with acetone, as shown by Karl Fischer moisture determinations on the products, but at a slower rate than occurred with methanol; no retention of ethyl acetate or acetone was found. This selectivity in retention of organic compounds during the transformation of α-lactose monohydrate to anhydrous α-lactose is different from that observed for freeze-dried lactose and other carbohydrates with these liquids (Flink and Karel, 1970) and for α_M with their vapors (Lee et al., 1975).

We have found a distinct difference between the anomeric forms of anhydrous lactose in retention of methanol. The stable α-lactose, α_S (Sharp, 1943), a hygroscopic α-lactose, α_H (Herrington, 1948), showed levels of 0.14 and 0.12 wt %, respectively, whereas no retention of methanol occurred with β-lactose (Buma and van der Veen, 1974). In the preparation of the last-named compound, the crystalline material is washed with hot (140 °C) glycerol followed by hot ethanol; we have shown by ^{1}H NMR examination that neither of these compounds is retained. However, when β-lactose was prepared from α-lactose monohydrate by reaction with potassium methoxide in methanol or ethanol (Parrish et al., 1978), the retention of these alcohols was 0.79 and 0.30 wt %, respectively (Table III). These values are similar to the values for α_M and α_E formed from α-lactose monohydrate. The β-lactose crystallized from hot water (Buma and van der Veen, 1974) had melting point 235 °C by DSC, whereas the melting point of β-lactose prepared by reaction of α-lactose monohydrate with potassium methoxide in methanol was 230 °C.

When β-lactose which had retained methanol was treated at 27 °C for 16 h with 90% aqueous methanol or 90% aqueous ethanol, no change in the level of retained methanol occurred and no ethanol was incorporated into the sample.

We have also measured the retention of methanol and ethanol by freeze-dried lactose (Flink and Karel, 1970), the values being 0.13 and 0.47 wt %, respectively; these values are lower than the value of 2.44 wt % reported by Flink and Karel (1970) for retention of 1-butanol by freeze-dried lactose, no data being given for methanol, ethanol, or 1-propanol. These data indicate that different mechanisms are involved for alcohol retention by freeze-dried and crystalline lactose.

The gas chromatography and ^{1}H NMR techniques gave comparable results for retention of alcohols by lactose. An advantage of the ^{1}H NMR method is that the mole ratio of any alcohol to lactose is obtained without having to know the concentration of the sample solution and without the need for standard calibration with each alcohol as required by the gas chromatographic method.

Factors Involved in Retention of Alcohols. The data shown in Tables II and III indicate that voids in the crystalline lactose structure which can be filled by methanol are available and that transformation of α-lactose monohydrate to α_M or β-lactose in the presence of methanol provides more void space than is available in α_S. The lower level of incorporation of ethanol, 1-propanol, and 1-butanol, relative to methanol, can be explained on the basis of molecular size and relative hydrophobicity considerations.

ACKNOWLEDGMENT

We thank V. H. Holsinger of this Center for assistance with the Karl Fischer moisture determinations.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, D.C., 1975.
Berlin, E., Kliman, P. G., Anderson, B. A., Pallansch, M. J., *Thermochim. Acta* **2**, 143 (1971).
Buma, T. J., van der Veen, H. K. C., *Neth. Milk Dairy J.* **28**, 175 (1974).
Flink, J., Karel, M., *J. Agric. Food Chem.* **18**, 295 (1970).
Herrington, B. L., Borden Award Address, National Meeting of the American Chemical Society, Portland, Oreg., 1948.
Lee, I., Nickerson, T. A., Bernhard, R. A., *J. Dairy Sci.* **58**, 319 (1975).
Lim, S. G., Nickerson, T. A., *J. Dairy Sci.* **56**, 843 (1973).
Menting, L. C., Hoogstad, B., *J. Food Sci.* **32**, 87 (1967).
Nickerson, T. A., Lim, S. G., *J. Dairy Sci.* **57**, 1320 (1974).
Parrish, F. W., Ross, K. D., Simpson, T. D., *Carbohydr. Res.*, in press (1978).
Ross, K. D., *J. Dairy Sci.* **61**, 152 (1978).
Schwartz, D. P., *Anal. Biochem.* **38**, 148 (1970).
Sharp, P. F., *U.S. Patent* 2319562 (1943).
Sondack, D. L., *Anal. Chem.* **44**, 888 (1972).